Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1763 (2006) 414–421
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Review

Mitochondrial morphology and protein import—A tight connection?

Diana Stojanovski, Michael Rissler, Nikolaus Pfanner, Chris Meisinger *

Institut für Biochemie und Molekularbiologie, Universität Freiburg, Hermann-Herder-Straße 7, D-79104 Freiburg, Germany

Received 13 January 2006; received in revised form 17 February 2006; accepted 22 February 2006
Available online 20 March 2006

Abstract

Although the field of mitochondrial protein import and assembly may have initially been viewed as a completely distinct area of investigation to that of mitochondrial morphology and dynamics, recent findings have noted a clear influence on organelle morphology by perturbations in protein import pathways. This review aims to provide an overview of the mitochondrial import machinery in context of the recent link between translocation components and organelle structure, in addition to conferring the questions and challenges that have surfaced due to these observations.

Keywords: Mitochondria; Protein import; Protein assembly; *Saccharomyces cerevisiae*

1. Introduction

The enclosure of mitochondria by a double membrane lipid bilayer reflects the organelle's prokaryotic ancestry. Through the course of evolution the mitochondrial α-proteobacterial ancestor displaced a large proportion of its genes to the host nuclear genome. Consequently, the greater part of mitochondrial proteins (about 1000 in the Baker's yeast *Saccharomyces cerevisiae*) are encoded by nuclear genes and after their synthesis on cytosolic ribosomes must be imported into the organelle [1–3]. The mitochondrial outer and inner membranes effectively serve as barriers for the maintenance and integrity of two soluble compartments, the mitochondrial intermembrane space (IMS) and mitochondrial matrix. The correct delivery and sorting of nuclear encoded mitochondrial precursors to each of these four compartments is pivotal in the maintenance of normal organelle function and undoubtedly organelle structure. However, in addition to being delivered to the correct submitochondrial location many proteins have to be further assembled into homo- or hetero-oligomeric structures, in order to fulfill their functions. Elegant translocation machineries within the outer and inner membranes in addition to translocation mediators within the organelle's soluble regions take on and execute these delicate tasks (Fig. 1) [4,5].

Recent studies at both the high throughput level and analysis of single proteins have revealed links between components involved in the biogenesis of mitochondrial preproteins and organelle morphology. Components of the mitochondrial protein import and assembly machinery that have been reported to influence mitochondrial morphology are shown in red in Figs. 2 and 3. These surfacing connections between what have so far remained independent fields raise the requirement for a careful dissection of direct and indirect effects on organelle structure not only by new but also previously established mediators.

2. Import across and into the outer membrane

The TOM (Translocase of the Outer Mitochondrial membrane) complex embodies the recognition site and central entry gate for the import of essentially all nuclear encoded mitochondrial proteins. In a collaborative manner, its constituents effectively transport components into or across the mitochondrial outer membrane. The TOM complex consists of seven different subunits: the channel forming unit Tom40, the receptor components Tom22, Tom20 and Tom70 and the small Tom proteins, Tom5, Tom6 and Tom7 (Fig. 2) [6–8]. The mitochondrial outer membrane houses components of the

Abbreviations: $\Delta\psi$, membrane potential; IMS, intermembrane space; PAM, presequence translocase-associated motor; SAM, sorting and assembly machinery; TIM, translocase of the inner membrane; TOM, translocase of the outer membrane

* Corresponding author. Tel.: +49 761 203 5287; fax: +49 761 203 5261.
E-mail address: christof.meisinger@biochemie.uni-freiburg.de (C. Meisinger).

0167-4889/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2006.02.008

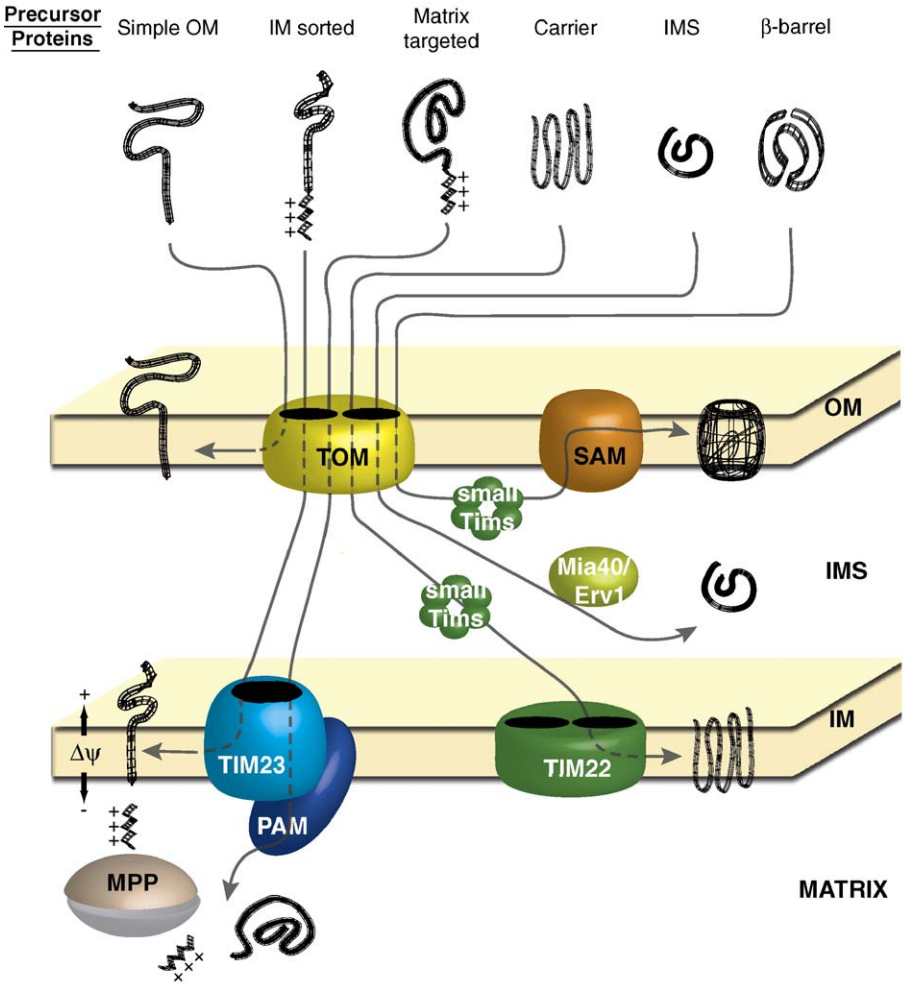

Fig. 1. Protein import pathways into mitochondria. The greater part of mitochondrial precursors are synthesized on cytosolic ribosomes and are subsequently imported into the organelle in a post-translational manner. Precursor proteins can be divided into at least six classes: (i) simple outer membrane (OM) proteins, (ii) inner membrane (IM) sorted, (iii) matrix targeted, (iv) carrier proteins of the inner membrane, (v) intermembrane space (IMS) targeted and (vi) outer membrane β-barrel precursors. Common to all precursors is their delivery to the organelle's central entry gate, the translocase of the outer membrane (TOM complex), upon which their import routes diverge depending on individual or multiple targeting signals contained within their primary structure. Simple OM proteins (defined by the presence of single α-helical transmembrane segments) can be directly imported into the OM with the aid of the TOM complex. Precursors possessing an N-terminal presequence include a number that require sorting into the IM and all matrix destined precursors. These precursors are sorted to the presequence translocase of the inner membrane (TIM23 complex) in a membrane potential ($\Delta\psi$) dependent manner. IM precursors are halted in the TIM23 translocon by the presence of a "stop transfer signal" and upon proteolytic removal of the presequence by the mitochondrial processing peptidase (MPP) are laterally released into the lipid bilayer. Complete translocation of precursors into the mitochondrial matrix is driven by the ATP-driven presequence translocase-associated motor (PAM complex). Upon entry into the matrix presequence removal mediated by MPP permits folding of matrix residents into their functional state. Hydrophobic inner membrane proteins possessing non-cleavable targeting elements, such as the carrier proteins, are guided through the aqueous IMS by complexes of small Tim proteins and delivered to and inserted into the inner membrane by the $\Delta\psi$-driven carrier translocase (TIM22 complex). Small cysteine rich IMS precursors are translocated through the TOM complex and their import is facilitated by the IMS-specific import components, consisting of Mia40 and Erv1. Outer membrane precursors with more complex topologies, such as β-barrel proteins, are translocated through the TOM complex into the IMS, where they are guided by the IMS small Tim proteins to the sorting and assembly machinery (SAM complex) for their integration into the OM.

fusion and fission machineries and is likely to be residence to many factors that govern organelle dynamics by promoting interactions with the cytoskeleton. The mechanism by which simple outer membrane precursors, that is, those possessing single α-helical transmembrane segments, are delivered to and integrated into the membrane remains an open question. It is clear that the TOM complex plays a role in the import of some outer membrane precursors, but whether the complex plays a universal role in the import and assembly of all outer membrane proteins remains unknown [9,10].

Precursor proteins that are translocated through the TOM complex are sorted to one of at least four alternative import routes governed by individual or multiple targeting signals within their primary structure (Fig. 1). For the integration of outer membrane proteins with more complex topologies belonging to the β-barrel structural family, such as Tom40 and porin, the TOM complex alone is not sufficient. These precursors additionally require the Sorting and Assembly Machinery (SAM complex) of the mitochondrial outer membrane for their successful integration and assembly into

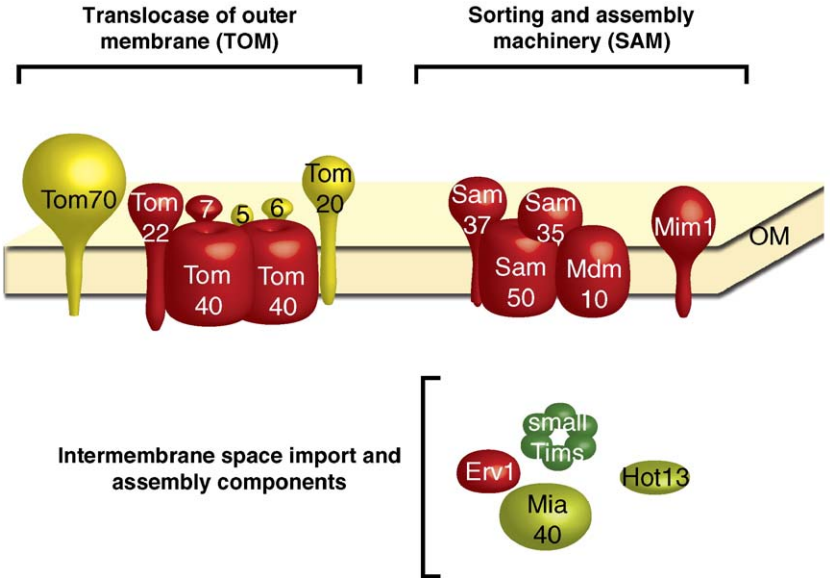

Fig. 2. Outer membrane and intermembrane space import and assembly components. The translocase of the outer membrane (TOM complex) consists of the central pore forming unit, Tom40, three receptor units, Tom22, Tom20 and Tom70, and the three small Toms, Tom5, Tom6 and Tom7. The sorting and assembly machinery (SAM complex) of the mitochondrial outer membrane (OM) is composed of the core subunit, Sam50, which collaborates with Sam35 and Sam37 to mediate the integration of β-barrel proteins into the OM. For the integration of Tom40 into the OM functioning of a further component, Mdm10, a previously defined regulator of mitochondrial morphology and inheritance, is required at the SAM complex. A further resident of the mitochondrial OM, Mim1, appears to be required for the assembly of a functional TOM complex. The intermembrane space (IMS) import and assembly machinery consists of the small Tim family members and the recently defined components Mia40, Erv1 and Hot13. Constituents of these machineries that have been shown to have an influence on organelle morphology upon their deletion or mutation (as detailed in text) are shown in red.

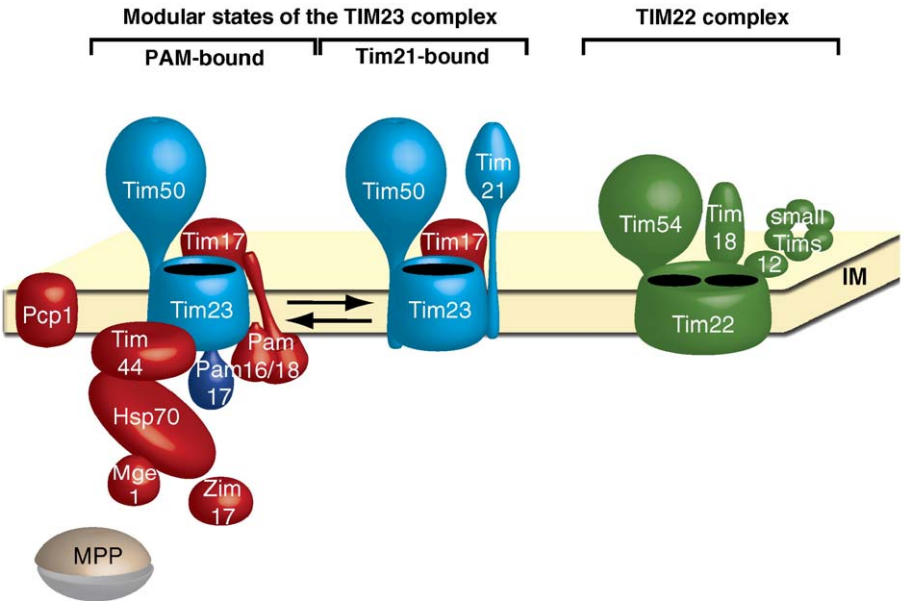

Fig. 3. Inner membrane import and assembly components. The presequence translocase of the inner membrane (TIM23 complex) can exist in two alternative states influenced by the presence or absence of the inner membrane constituent, Tim21. The absence of Tim21 permits association of the presequence translocase-associated motor (PAM complex) with the TIM23 complex (left). The PAM complex contains as its central player mtHsp70, which is transiently anchored at the translocase by Tim44 and requires the additional components Pam18, Pam17, Pam16 and Mge1 for promotion and maintenance of several ATP-dependent cycles. A PAM-bound TIM23 complex is competent for the import of matrix precursors into the matrix that is followed by presequence removal mediated by the mitochondrial processing peptidase (MPP). The PAM-bound TIM23 complex is exploited for the successful integration of Mgm1 into the inner membrane, upon which cleavage by the inner membrane protease Pcp1 gives rise to an alternative isoform of the inner membrane dynamin member. An additional constituent, Zim17, is a matrix heat shock protein with a specific function in preventing the aggregation of mitochondrial Hsp70 chaperones. A Tim21-bound complex (middle), consisting of Tim23, Tim50, Tim17 and Tim21 is competent in the sorting of inner membrane precursors. The carrier translocase of the inner membrane (TIM22 complex) consists of the three membrane integrated components, Tim22, Tim54 and Tim18 and the peripherally associated Tim12. Constituents of these machineries that have been shown to have an influence on organelle morphology upon their deletion or mutation (as detailed in text) are shown in red.

functional complexes [11]. The SAM complex is composed of three core constituents, Sam37 (Mas37), the first identified component of the complex [11], and the two essential proteins Sam50 (Tob55/Omp85) and Sam35 (Tob38/Tom38) (Fig. 2) [12–17]. Yeast mutants of either of these components display specific defects in the assembly of β-barrel proteins into the outer membrane. Interestingly, the import pathway of β-barrel precursors was revealed to encompass an initial translocation into the IMS via the TOM complex, upon which with the aid of the IMS small Tim proteins they can be delivered to the SAM complex for their integration into the outer membrane (Figs. 1 and 2) [18–20]. From an evolutionary perspective, this is an intriguing finding given that the central component of the SAM complex, Sam50, displays homology to the bacterial outer membrane protein Omp85, which functions in β-barrel protein export from the periplasm into the outer membrane [21]. This infers a conserved ancestral mechanism for the integration of β-barrel precursors into the outer membrane and further highlights the prokaryotic origin of mitochondria.

3. Import into the mitochondrial IMS

Small IMS precursors were initially believed to utilize a relatively simple import pathway, which relied only on the TOM complex for their translocation into the IMS [22]. However, two recently identified components of a specific mitochondrial IMS import machinery, Mia40 and Erv1, have shed much light into the biogenesis of small cysteine rich IMS precursors and indeed, the import pathway of these precursors is much more complex than may have initially been envisaged. Depletion of endogenous Mia40 and Erv1, or disruption of their function through mutation, leads to a specific block in the import of small cysteine rich IMS proteins, such as members of the small Tim family, Cox17, and Cox19 without affecting other import pathways [23–28]. Both Mia40 and Erv1, like most IMS proteins contain characteristic cysteine motifs, which in the case of Mia40 appear to be essential for its function [24]. Mia40 appears to play a role in early steps of IMS import, aiding the complete translocation of precursors across the outer membrane into the IMS [23]. IMS substrate and Mia40 import intermediates are decreased in the presence of reducing agents [23,25], but stabilized in the presence of CuCl2, which induces disulfide bond cross-linking [23]. These observations suggest the formation of disulfide bridges play a role in the process of IMS protein import, which would rely on the presence of sulfhydryl oxidation reactions in the IMS. Indeed, Erv1 was initially shown to function as a flavin adenine dinucleotide (FAD)-dependent sulfhydryl oxidase in the mitochondrial IMS, but no substrates for the protein had been reported [29,30]. Interactions between Mia40 and Erv1 have been revealed and it is now clear that Mia40 is a physiological substrate for Erv1 [25,26], and that these two constituents contribute to a disulfide relay system within the mitochondrial IMS, although the precise molecular detail of how this is executed awaits elucidation.

4. Import through and into the inner membrane

4.1. The presequence translocase: TIM23 complex

Preproteins with the classical mitochondrial N-terminal presequence are typically destined for the mitochondrial matrix (Fig. 1). After their translocation and release from the TOM complex, these preproteins are directed to the inner membrane presequence translocase, the TIM23 complex. The TIM23 complex consists of the integral membrane components, Tim23, Tim50 and Tim17 [31,32], and the recently identified Tim21 (Fig. 3) [33]. An exposed IMS domain of Tim50 mediates the initial interaction of preproteins with the presequence translocase [34–36], upon which preproteins are translocated into the import channel formed by Tim23 in a manner reliant on a membrane potential ($\Delta\psi$) [37]. Presequence containing precursors that possess a further "stop transfer signal" can be sorted directly into the inner membrane by the TIM23 complex. However, precursors that require complete translocation into the mitochondrial matrix require an additional driving force that is provided by the presequence translocase-associated protein import motor (PAM complex), which contains matrix mitochondrial Hsp70 (mtHsp70) as its central player. The PAM complex permits several ATP-dependent cycles and successive binding of mtHsp70 to the incoming precursor. The peripherally associated Tim44 appears to play a role in the loading of mtHsp70 to the precursor protein in transit, whilst the ATPase activity of the chaperone is stimulated by the integral co-chaperone, Pam18 (Tim14), whose association with the presequence translocase is modulated by a further component, Pam16 (Tim16) [38–40]. Eventually, ADP-ATP exchange is mediated by Mge1, which prepares mtHsp70 for a further round of cycling. Upon translocation proteolytic removal of the presequence by the mitochondrial processing peptidase (MPP) results in the liberation and successful delivery of the mature protein to its correct location, the mitochondrial matrix. Depending on the sorting signals in preproteins, the TIM23 complex switches between two modular states [33]: a PAM-free TIM23 complex containing Tim21 is competent for the sorting of preproteins into the inner membrane (Fig. 3, middle), whilst a PAM-bound TIM23 complex lacking Tim21 is required for protein translocation into the matrix (Fig. 3, left).

The importance in maintaining mtHsp70's structure and function has been reiterated with the recent characterization of Zim17 (Tim15/Hep1) [41–44]. Zim17 has been revealed as a matrix heat shock protein with a specific role in preventing the aggregation of Hsp70 chaperones, including mtHsp70 [43,44]. The presence of a chaperone to monitor the structure and function of further chaperone components reaffirms the importance in maintaining an operational protein folding machinery for mitochondrial function.

4.2. The carrier pathway: TIM22 complex

Polytopic inner membrane proteins represented by model substrates such as AAC (ADP/ATP Carrier) lack the classical N-terminal presequence, but instead contain internal cryptic

targeting signals (Fig. 1). Upon translocation through the Tom40 pore, these largely hydrophobic precursors are guided through the aqueous IMS by chaperone-like complexes of small Tim proteins to an alternative translocase of the inner membrane, the TIM22 complex (Fig. 1). The TIM22 complex consists of at least three membrane-integrated components, Tim18, Tim22 and Tim54, and the peripherally associated constituent Tim12 (Fig. 3) [45–47]. In a $\Delta\psi$-dependent manner the TIM22 complex facilitates the insertion of carrier proteins into the inner membrane.

5. A link between mitochondrial import and organelle morphology

5.1. Outer membrane components

The search for new components of the SAM complex provided a solid and unexpected link between the fields of mitochondrial protein import/assembly and mitochondrial morphology, with the surprising elution of Mdm10 from a Sam37 affinity column (Fig. 2) [48]. In 1994, Sogo and Yaffe reported on the identification of a mitochondrial outer membrane protein, Mdm10, which in a non-functional form induced rearrangements in mitochondrial morphology from the normally extended tubular rods into giant spherical mitochondria. Cells harboring the mutant form of Mdm10 additionally displayed a clear defect in mitochondrial transmission to the bud [49]. What could a mitochondrial distribution and morphology factor be doing in association with outer membrane protein assembly factors? It is now clear that unlike the other SAM components that do not discriminate on the β-barrel substrates whose assembly they facilitate, Mdm10 plays a specific role in the assembly of the TOM complex. More specifically, Mdm10 appears to promote association of Tom40 with Tom22, Tom6 and Tom7, permitting the final stages in the assembly of the essential outer membrane translocon [48].

These observations raised questions into the primary function of Mdm10, that is, as a protein assembly factor or mitochondrial morphology factor. Could the morphology phenotype seen in Mdm10 mutants be due to pleiotropic effects induced as a consequence of inefficient TOM complex assembly and ultimately impaired protein import? If so, one would expect mutants of TOM and SAM components to give rise to similar aberrant mitochondrial morphologies analogous to those seen in the *mdm10* mutant. Indeed, *tom40* mutant cells in addition to cells harboring mutant forms of Sam50, Sam37 and Sam35 display condensed giant mitochondria, inferring alterations in mitochondrial morphology in *mdm10* mutants are likely secondary [48,50]. Thus, the SAM complex plays a pivotal role in the assembly of outer membrane proteins, in particular the essential TOM complex, which in turn plays a significant role in the maintenance of steady-state organelle morphology by permitting proper import of morphology factors.

However, *mdm10* mutants also displayed a clear segregation defect [49], and it is conceivable that Mdm10 could undertake a dual role, that is, functions in protein assembly in addition to regulating mitochondrial distribution and inheritance. The co-immunoprecipitation of Mdm10 with two other proteins involved in regulation of mitochondrial morphology and inheritance, Mmm1 and Mdm12, supports this notion [51]. Mutants of Sam37 and Sam50 also display a clear defect in the distribution of mitochondria to the bud while *tom40* mutant cells only show a minor defect in distribution of mitochondria [48]. One can postulate that this could be due to one of the following reasons: (i) individual or multiple components of the SAM complex undertake dual roles in both protein assembly and organelle distribution, (ii) the SAM complex is associated with or stabilizes components that govern organelle morphology and distribution, such as components that bridge an interaction between the mitochondria and the cytoskeleton or (iii) the SAM complex alone is involved in the import of mitochondrial morphology and distribution mediators. This latter point is indeed an exciting prospect, and if true would infer the presence of a second outer membrane translocation machinery, which for certain substrates has no reliance on the TOM complex. The recent finding that the TOM complex bears no apparent role in the association of Sam37 with mitochondria supports the potential existence of such a pathway [52]. As we gain further perspectives into the mitochondrial inheritance machinery the connection between this process and the SAM complex may be realized.

In addition to *tom40* mutants, two additional components of the TOM complex, Tom7 and Tom22 have been found to be required for normal organelle morphology in *S. cerevisiae* (Fig. 2). Tom7 was discovered in a systematic screen of deletion mutants to possess an effect on organelle morphology, since cells lacking Tom7 display aggregated mitochondria [53]. Tom22 was subsequently revealed in a systematic screen of a yeast strain collection harboring essential genes under the control of a regulated promoter [50]. Upon depletion of Tom22, the alterations in mitochondrial morphology were comparable to those seen for *tom7Δ* mutants, that is, a distinct aggregation of the mitochondrial network. The alterations in organelle morphology observed for both *tom7* and *tom22* mutants were reasoned to be secondary effects as a consequence of defects in the import of mitochondrial morphology factors [50]. This is most likely the case, in particular for Tom22 since it possesses the major receptor function within the TOM complex and is required for the assembly of the TOM components into a multimeric complex [8,18,48]. However, Tom7 is an interesting candidate since unpublished data from our group suggests that morphology defects appear to be more pronounced upon its deletion relative to the other small Toms, Tom5 and Tom6. Tom5 possesses functions in transfer of preproteins [54], whereas Tom6 and Tom7 seem to play counteractive roles in mediating interactions between TOM subunits [55]. A role of the small Toms in TOM complex dynamics is evident, but the different impact on organelle morphology by the alternative components may suggest the phenotype induced in *tom7Δ* mutants is more than just a secondary effect due to defects in protein import.

The problem of separating primary and secondary functions is also raised by the recent identification of the outer membrane

protein Mim1 (Tom13). Depletion of Mim1 leads to defects in both, protein assembly [17,56] and mitochondrial morphology [50]. Further studies will be needed to analyze the molecular function of Mim1 and its potential interactions with assembly or morphology components.

5.2. IMS components

Prior to its recently defined role as a component of the specific IMS import machinery [25–27], Erv1 had been the subject of investigations into its potential role in mitochondrial morphology and distribution [57]. Although at the time of this analysis, the cellular location and function of Erv1 were questionable, a temperature-sensitive mutant displayed clear alterations in mitochondrial structure. Ultra-structural analysis additionally revealed changes in cristae formation. No reports have as yet detailed alterations in organelle morphology as a consequence of impaired Mia40 function. However, mitochondria possessing defective Erv1 additionally display a clear defect in the assembly of Tim9 into the Tim9–Tim10 heterohexameric complex, supporting a role for Erv1 in protein complex assembly in addition to its role in IMS protein import [26]. It is therefore a possibility that Erv1 assists in the import and assembly of morphology candidates or is required for molecular regulators that subsequently aid in the import and assembly of morphology factors, explaining the morphology defects detailed in the early analysis of the protein (Fig. 2).

5.3. The inner membrane translocons

Of the two inner membrane translocons, the TIM23 complex and the TIM22 complex, it appears that only components of the TIM23 complex and its associated motor, the PAM complex, have an effect on organelle morphology upon their down-regulation or mutation (Fig. 3). Although components of the carrier pathway have been analyzed, their effect on organelle morphology appears minimal [50,53]. Depletion of the central component, Tim22, displays no effect, most likely inferring no morphology regulators exploit the carrier pathway for their import and integration into the inner membrane [50,58]. Alternatively, down-regulation of the presequence translocase essential components, Tim17 and Tim44, results in clear morphological defects with the mitochondria forming characteristic aggregates [58]. Pam18 and Mge1, cofactors of the presequence translocase-associated motor, have also been shown to induce alterations in organelle morphology [50,58].

Mutants of mtHsp70 cause an aggregation of the mitochondrial network, which has been reported to be independent of alterations in mitochondrial import pathways [59]. Kawai and colleagues suggest the mitochondrial matrix may be resident to at least one candidate with a role in organelle morphology, which has a dependence on the mtHsp70 system for its function, this, however, awaits verification. Mutants of Zim17 display a fragmentation of the mitochondrial network, but also possess defects in protein import [41–44]. Generation of a conditional zim17 mutant allowed for primary and secondary effects in the mutant to be separated. This analysis revealed aggregation of mitochondrial Hsp70 chaperones was the primary effect with the additional alterations in organelle morphology and effects on protein import surfacing after its establishment [44].

5.4. Alternative topogenesis pathway

The dependence of mitochondrial morphology factors on a functional import apparatus has probably been mostly realized for the inner membrane dynamin family member, Mgm1. Mgm1 possesses unique processing properties that gives rise to two isoforms of the protein (large, l-Mgm1 and small, s-Mgm1). Interestingly, both isoforms of Mgm1 appear to play a role in the regulation of mitochondrial morphology, with their absence inducing a fragmentation of the mitochondrial network and loss of mtDNA [60,61]. Analysis into the mechanism by which both isoforms are regulated has provided insights into the biogenesis of Mgm1, and its heavy reliance on functional import machinery, in particular a functional PAM complex (Fig. 3) [57,60–63]. The inner membrane potential provides the driving force for translocation of the Mgm1 presequence into the mitochondrial matrix, and the precursor's first transmembrane segment behaves as a stop transfer signal trapping the full-length precursor within the TIM23 translocon. Subsequent processing of the presequence by MPP, results in the generation of l-Mgm1 that can be laterally released into the inner membrane. For generation of s-Mgm1, the first transmembrane segment is further translocated into the mitochondrial matrix by the ATP driven PAM complex, and the second hydrophobic stretch is laterally released into the inner membrane which is suspected to be cleaved by the rhomboid protease Pcp1. Therefore, the relative ratio of l-Mgm1 to that of s-Mgm1 appears to be governed by the ATP levels in the matrix and ultimately the presequence translocase-associated motor [58].

Mitochondria lacking Pcp1 display similar morphological alterations to mitochondria from *mgm1Δ*, and expression of s-Mgm1 can partially complement the mitochondrial morphology phenotype in *pcp1Δ* cells [60]. Although it is clear that Mgm1 processing is abolished in absence of Pcp1, it cannot be excluded that the role of Pcp1 in Mgm1 processing is indirect, e.g., by activating another yet unknown protease, since a direct interaction between the two has not as yet been demonstrated.

6. Concluding remarks

The functioning of Mdm10 as a protein assembly factor raises the possibility that other factors implicated in the maintenance of organelle morphology may also be involved in the assembly of functional complexes. As the field of mitochondrial morphology and dynamics expands at the accelerated rate that we are witnessing, we may need to implement some caution in the assigning of candidates as morphology regulators. From the information presented in this review, it is clear that disturbances in protein import into mitochondria can have a clear effect on organelle structure. Our knowledge into the primary function of many of these candidates allows us to in many instances dismiss the morphology defects as secondary due to impaired protein

import. However, now when assigning functions to novel protein candidates with previously undefined functions, are alterations in mitochondrial morphology enough to designate these candidates as regulators of organelle morphogenesis? Under such circumstances it may be necessary to exploit additional biochemical analyses, such as surveys into functional protein import as additional controls. We may also consider the possibility of the reverse situation where organelle structure may have an effect on the regulation and efficiency of import. Work from our laboratory however, reveals no impact on protein import and assembly in mutants of established morphology factors, such as Ugo1, Fzo1, Mgm1, Dnm1, Fis1, Mdv1 and others where the structure of the organelle is clearly altered (Meisinger, C., Pfannschmidt, S., Pfanner, N., Wiedemann, N., unpublished data). Such key controls verify the specificity of these components in regulating organelle morphology.

The story of Mgm1 has provided clear insights into the knowledge we can gain by exploring additional aspects such as protein import pathways of morphology candidates. It is clear that crosstalk between the processes of mitochondrial protein import and processes regulating organelle structure are essential in the biogenesis and normal functioning of mitochondria. Our task as researchers in these fields of study is to now decipher how interrelated these aspects are in the maintenance of a dynamic mitochondrial population.

Acknowledgments

Work of the authors' laboratory was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 388, Max Planck Research Award, the Alexander von Humboldt Foundation, Bundesministerium für Bildung und Forschung, Gottfried Wilhelm Leibniz-Program and the Fonds der Chemischen Industrie.

References

[1] A. Sickmann, J. Reinders, Y. Wagner, C. Joppich, R. Zahedi, H.E. Meyer, B. Schönfisch, I. Perschil, A. Chacinska, B. Guiard, P. Rehling, N. Pfanner, C. Meisinger, The proteome of *Saccharomyces cerevisiae* mitochondria, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13207–13212.

[2] G. Schatz, B. Dobberstein, Common principles of protein translocation across membranes, Science 271 (1996) 1519–1526.

[3] W. Neupert, Protein import into mitochondria, Annu. Rev. Biochem. 66 (1997) 863–917.

[4] P. Rehling, K. Brandner, N. Pfanner, Mitochondrial import and the twin-pore translocase, Nat. Rev. Mol. Cell Biol. 5 (2004) 519–530.

[5] C.M. Koehler, New developments in mitochondrial assembly, Annu. Rev. Cell Dev. Biol. 20 (2004) 309–335.

[6] K. Hill, K. Model, M.T. Ryan, K. Dietmeier, F. Martin, R. Wagner, N. Pfanner, Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins, Nature 395 (1998) 516–521.

[7] K.P. Künkele, S. Heins, M. Dembowski, F.E. Nargang, R. Benz, M. Thieffry, J. Walz, R. Lill, S. Nussberger, W. Neupert, The preprotein translocation channel of the outer membrane of mitochondria, Cell 93 (1998) 1009–1019.

[8] S. van Wilpe, M.T. Ryan, K. Hill, A.C. Maarse, C. Meisinger, J. Brix, P.J.T. Dekker, M. Moczko, R. Wagner, M. Meijer, B. Guiard, A. Hönlinger, N. Pfanner, TOM22 is a multifunctional organizer of the mitochondrial preprotein translocase, Nature 401 (1999) 485–489.

[9] D. Rapaport, Finding the right organelle. Targeting signals in mitochondrial outer membrane proteins, EMBO Rep. 4 (2003) 948–952.

[10] N. Pfanner, N. Wiedemann, C. Meisinger, T. Lithgow, Assembling the mitochondrial outer membrane, Nat. Struct. Mol. Biol. 11 (2004) 1044–1048.

[11] N. Wiedemann, V. Kozjak, A. Chacinska, B. Schönfisch, S. Rospert, M.T. Ryan, N. Pfanner, C. Meisinger, Machinery for protein sorting and assembly in the mitochondrial outer membrane, Nature 424 (2003) 565–571.

[12] V. Kozjak, N. Wiedemann, D. Milenkovic, C. Lohaus, H.E. Meyer, B. Guiard, C. Meisinger, N. Pfanner, An essential role of Sam50 in the protein sorting and assembly machinery of the mitochondrial outer membrane, J. Biol. Chem. 278 (2003) 48520–48523.

[13] S.A. Paschen, T. Waizenegger, T. Stan, M. Preuss, M. Cyrklaff, K. Hell, D. Rapaport, W. Neupert, Evolutionary conservation of biogenesis of beta-barrel membrane proteins, Nature 426 (2003) 862–866.

[14] I. Gentle, K. Gabriel, P. Beech, R. Waller, T. Lithgow, The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria, J. Cell Biol. 164 (2004) 19–24.

[15] D. Milenkovic, V. Kozjak, N. Wiedemann, C. Lohaus, H.E. Meyer, B. Guiard, N. Pfanner, C. Meisinger, Sam35 of the mitochondrial protein sorting and assembly machinery is a peripheral outer membrane protein essential for cell viability, J. Biol. Chem. 279 (2004) 22781–22785.

[16] T. Waizenegger, S.J. Habib, M. Lech, D. Mokranjac, S.A. Paschen, K. Hell, W. Neupert, D. Rapaport, Tob38, a novel essential component in the biogenesis of beta-barrel proteins of mitochondria, EMBO Rep. 5 (2004) 704–709.

[17] D. Ishikawa, H. Yamamoto, Y. Tamura, K. Moritoh, T. Endo, Two novel proteins in the mitochondrial outer membrane mediate beta-barrel protein assembly, J. Cell Biol. 166 (2004) 621–627.

[18] K. Model, C. Meisinger, T. Prinz, N. Wiedemann, K.N. Truscott, N. Pfanner, M.T. Ryan, Multi-step assembly of the protein import channel of the mitochondrial outer membrane, Nat. Struct. Biol. 8 (2001) 361–370.

[19] S.C. Hoppins, F.E. Nargang, The Tim8–Tim13 complex of *Neurospora crassa* functions in the assembly of proteins into both mitochondrial membranes, J. Biol. Chem. 279 (2004) 12396–12405.

[20] N. Wiedemann, K.N. Truscott, S. Pfannschmidt, B. Guiard, C. Meisinger, N. Pfanner, Biogenesis of the protein import channel Tom40 of the mitochondrial outer membrane: intermembrane space components are involved in an early stage of the assembly pathway, J. Biol. Chem. 279 (2004) 18188–18194.

[21] R. Voulhoux, M.P. Bos, J. Geurtsen, M. Mols, J. Tommassen, Role of a highly conserved bacterial protein in outer membrane protein assembly, Science 299 (2003) 262–265.

[22] T. Lutz, W. Neupert, J.M. Herrmann, Import of small Tim proteins into the mitochondrial intermembrane space, EMBO J. 22 (2003) 4400–4408.

[23] A. Chacinska, S. Pfannschmidt, N. Wiedemann, V. Kozjak, L.K. Sanjuán-Szklarz, A. Schulze-Specking, K.N. Truscott, B. Guiard, C. Meisinger, N. Pfanner, Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins, EMBO J. 23 (2004) 3735–3746.

[24] M. Naoé, Y. Ohwa, D. Ishikawa, C. Ohshima, S. Ishikawa, H. Yamamoto, T. Endo, Identification of Tim40 that mediates protein sorting to the mitochondrial intermembrane space, J. Biol. Chem. 279 (2004) 47815–47821.

[25] N. Mesecke, N. Terziyska, C. Kozany, F. Baumann, W. Neupert, K. Hell, J.M. Herrmann, A disulfide relay system in the intermembrane space of mitochondria that mediates protein import, Cell 121 (2005) 1059–1069.

[26] M. Rissler, N. Wiedemann, S. Pfannschmidt, K. Gabriel, B. Guiard, N. Pfanner, A. Chacinska, The essential mitochondrial protein Erv1 cooperates with Mia40 in biogenesis of intermembrane space proteins, J. Mol. Biol. 353 (2005) 485–492.

[27] S. Allen, V. Balabanidou, D.P. Sideris, T. Lisowsky, K. Tokatlidis, Erv 1 mediates the Mia40-dependent protein import pathway and provides a functional link to the respiratory chain by shuttling electrons to cytochrome *c*, J. Mol. Biol. 353 (2005) 937–944.

[28] J.M. Herrmann, K. Hell, Chopped, trapped or tacked-protein translocation into the IMS of mitochondria, Trends Biochem. Sci. 30 (2005) 205–211.
[29] J. Lee, G. Hofhaus, T. Lisowsky, Erv1p from *Saccharomyces cerevisiae* is a FAD-linked sulfhydryl oxidase, FEBS Lett. 477 (2000) 62–66.
[30] H. Lange, T. Lisowsky, J. Gerber, U. Mühlenhoff, G. Kispal, R. Lill, An essential function of the mitochondrial sulfhydryl oxidase Erv1p/ALR in the maturation of cytosolic Fe/S proteins, EMBO Rep. 2 (2001) 715–720.
[31] R.E. Jensen, A.E. Johnson, Opening the door to mitochondrial protein import, Nat. Struct. Biol. 8 (2001) 1008–1010.
[32] N. Pfanner, A. Geissler, Versatility of the mitochondrial protein import machinery, Nat. Rev. Cell Biol. 2 (2001) 339–349.
[33] A. Chacinska, M. Lind, A.E. Frazier, J. Dudek, C. Meisinger, A. Geissler, A. Sickmann, H.E. Meyer, K.N. Truscott, B. Guiard, N. Pfanner, P. Rehling, Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17, Cell 120 (2005) 817–829.
[34] A. Geissler, A. Chacinska, K.N. Truscott, N. Wiedemann, K. Brandner, A. Sickmann, H.E. Meyer, C. Meisinger, N. Pfanner, P. Rehling, The mitochondrial presequence translocase: an essential role of Tim50 in directing preproteins to the import channel, Cell 111 (2002) 507–518.
[35] H. Yamamoto, M. Esaki, T. Kanamori, Y. Tamura, S. Nishikawa, T. Endo, Tim50 is a subunit of the TIM23 complex that links protein translocation across the outer and inner mitochondrial membranes, Cell 111 (2002) 519–528.
[36] D. Mokranjac, S.A. Paschen, C. Kozany, H. Prokisch, S.C. Hoppins, F.E. Nargang, W. Neupert, K. Hell, Tim50, a novel component of the TIM23 protein translocase of mitochondria, EMBO J. 22 (2003) 816–825.
[37] K.N. Truscott, P. Kovermann, A. Geissler, A. Merlin, M. Meijer, A.J. Driessen, J. Rassow, N. Pfanner, R. Wagner, A presequence- and voltage-sensitive channel of the mitochondrial preprotein translocase formed by Tim23, Nat. Struct. Biol. 8 (2001) 1074–1082.
[38] A.E. Frazier, J. Dudek, B. Guiard, W. Voos, Y. Li, M. Lind, C. Meisinger, A. Geissler, A. Sickmann, H.E. Meyer, V. Bilanchone, M.G. Cumsky, K.N. Truscott, N. Pfanner, P. Rehling, Pam16 has an essential role in the mitochondrial protein import motor, Nat. Struct. Mol. Biol. 11 (2004) 226–233.
[39] C. Kozany, D. Mokranjac, M. Sichting, W. Neupert, K. Hell, The J domain-related cochaperone Tim16 is a constituent of the mitochondrial TIM23 preprotein translocase, Nat. Struct. Mol. Biol. 11 (2004) 234–241.
[40] P.R. D'Silva, B. Schilke, W. Walter, E.A. Craig, Role of Pam16's degenerate J domain in protein import across the mitochondrial inner membrane, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 12419–12424.
[41] L. Burri, K. Vascotto, S. Fredersdorf, R. Tiedt, M.N. Hall, T. Lithgow, Zim17, a novel zinc finger protein essential for protein import into mitochondria, J. Biol. Chem. 279 (2004) 50243–50249.
[42] H. Yamamoto, T. Momose, Y. Yatsukawa, C. Ohshima, D. Ishikawa, T. Sato, Y. Ohwa, T. Endo, Identification of a novel member of yeast mitochondrial Hsp70-associated motor and chaperone protein that facilitates protein translocation across the inner membrane, FEBS Lett. 579 (2005) 507–511.
[43] M. Sichting, D. Mokranjac, A. Azem, W. Neupert, K. Hell, Maintenance of structure and function of mitochondrial Hsp70 chaperones requires the chaperone Hep1, EMBO J. 24 (2005) 1046–1056.
[44] L.K. Sanjuán Szklarz, B. Guiard, M. Rissler, N. Wiedemann, V. Kozjak, M. van der Laan, C. Lohaus, K. Marcus, H.E. Meyer, A. Chacinska, N. Pfanner, C. Meisinger, Inactivation of the mitochondrial heat shock protein Zim17 leads to aggregation of matrix Hsp70s followed by pleiotropic effects on morphology and protein biogenesis, J. Mol. Biol. 351 (2005) 206–218.
[45] P. Rehling, K. Model, K. Brandner, P. Kovermann, A. Sickmann, H.E. Meyer, W. Kühlbrandt, R. Wagner, K.N. Truscott, N. Pfanner, Protein insertion into the mitochondrial inner membrane by a twin-pore translocase, Science 299 (2003) 1747–1751.
[46] C.M. Koehler, The small Tim proteins and the twin Cx3C motif, Trends Biochem. Sci. 29 (2004) 1–4.
[47] N. Wiedemann, A.E. Frazier, N. Pfanner, The protein import machinery of mitochondria, J. Biol. Chem. 279 (2004) 14473–14476.
[48] C. Meisinger, M. Rissler, A. Chacinska, D. Milenkovic, V. Kozjak, B. Schönfisch, C. Lohaus, H.E. Meyer, M.P. Yaffe, B. Guiard, N. Wiedemann, N. Pfanner, The mitochondrial morphology protein Mdm10 functions in assembly of the preprotein translocase of the outer membrane, Dev. Cell 7 (2004) 61–71.
[49] L.F. Sogo, M.P. Yaffe, Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane, J. Cell Biol. 126 (1994) 1361–1373.
[50] K. Altmann, B. Westermann, Role of essential genes in mitochondrial morphogenesis in Saccharomyces cerevisiae, Mol. Biol. Cell 16 (2005) 5410–5417.
[51] I.R. Boldogh, D.W. Nowakowski, H.C. Yang, H. Chung, S. Karmon, P. Royes, L.A. Pon, A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery, Mol. Biol. Cell 14 (2003) 4618–4627.
[52] S.J. Habib, T. Waizenegger, M. Lech, W. Neupert, D. Rapaport, Assembly of the TOB complex of mitochondria, J. Biol. Chem. 280 (2005) 6434–6440.
[53] K.S. Dimmer, S. Fritz, F. Fuchs, M. Messerschmitt, N. Weinbach, W. Neupert, B. Westermann, Genetic basis of mitochondrial function and morphology in *Saccharomyces cerevisiae*, Mol. Biol. Cell 13 (2002) 847–853.
[54] K. Dietmeier, A. Hönlinger, U. Bömer, P.J. Dekker, C. Eckerskorn, F. Lottspeich, M. Kübrich, N. Pfanner, Tom5 functionally links mitochondrial preprotein receptors to the general import pore, Nature 388 (1997) 195–200.
[55] P.J.T. Dekker, M.T. Ryan, J. Brix, H. Müller, A. Hönlinger, N. Pfanner, Preprotein translocase of the outer mitochondrial membrane: molecular dissection and assembly of the general import pore complex, Mol. Cell. Biol. 18 (1998) 6515–6524.
[56] T. Waizenegger, S. Schmitt, J. Zivkovic, W. Neupert, D. Rapaport, Mim1, a protein required for the assembly of the TOM complex of mitochondria, EMBO Rep. 6 (2005) 57–62.
[57] D. Becher, J. Kricke, G. Stein, T. Lisowsky, A mutant for the yeast scERV1 gene displays a new defect in mitochondrial morphology and distribution, Yeast 15 (1999) 1171–1181.
[58] M. Herlan, C. Bornhovd, K. Hell, W. Neupert, A.S. Reichert, Alternative topogenesis of Mgm1 and mitochondrial morphology depend on ATP and a functional import motor, J. Cell Biol. 165 (2004) 167–173.
[59] A. Kawai, S. Nishikawa, A. Hirata, T. Endo, Loss of the mitochondrial Hsp70 functions causes aggregation of mitochondria in yeast cells, J. Cell Sci. 114 (2001) 3565–3574.
[60] M. Herlan, F. Vogel, C. Bornhovd, W. Neupert, A.S. Reichert, Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA, J. Biol. Chem. 278 (2003) 27781–27788.
[61] H. Sesaki, S.M. Southard, A.E. Hobbs, R.E. Jensen, Cells lacking Pcp1p/Ugo2p, a rhomboid-like protease required for Mgm1 processing, lose mtDNA and mitochondrial structure in a Dnm1p-dependent manner, but remain competent for mitochondria fusion, Biochem. Biophys. Res. Commun. 308 (2003) 276–283.
[62] K. Esser, B. Tursun, M. Ingenhoven, G. Michaelis, E. Pratje, A novel two-step mechanism for removal of a mitochondrial signal sequence involves the mAAA complex and the putative rhomboid protease Pcp1, J. Mol. Biol. 323 (2002) 835–843.
[63] G.A. McQuibban, S. Saurya, M. Freeman, Mitochondrial membrane remodeling regulated by a conserved rhomboid protease, Nature 423 (2003) 537–541.